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Characterisation of Novel Extracellular Molecular Chaperones and Their
Effects on Amyloid Formation.

A thesis submitted in fulfilment of the requirements for the award of the degree

Doctor of Philosophy

from

the UNIVERSITY of WOLLONGONG

by

Justin J. Yerbury, BSc, BCom

School of Biological Sciences
University of Wollongong, Wollongong, Australia

2007

Certification

I, Justin J. Yerbury, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Justin J. Yerbury

10 January 2008

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List of Abbreviations

A β	amyloid β peptide
ALS	amyotrophic lateral sclerosis
ADP	adenosine diphosphate
ATP	adenosine triphosphate
b	biotin
BAG-1	Bcl-2-associated anti-death gene-1
bisANS	4,4'-bis(1-anilinonaphthalene-8-sulfonate)
BSA	bovine serum albumin
Calc	calcitonin
κ -cas	κ -casein
cc β	coiled-coil β peptide
CD	circular dichroism
CDS	clusterin depleted serum
CHIP	C-terminus Hsp70 interacting protein
CON	control
CPK	creatine phosphokinase
CS	citrate synthase
CSF	cerebrospinal fluid
Da	daltons
DDS	double depleted serum
DF	disturbing factors
DLS	dynamic light scattering
DNA	deoxyribonucleic acid
DNP	2,4-dinitrophenol
ECL	enhanced chemiluminescence
EDTA	ethylenediamine tetraacetic acid
FBS	foetal bovine serum
FITC	fluorescein isothiocyanate
fMLP	formyl-met-Leu-Phe
GSH	reduced glutathione

GST	glutathione-S-transferase
Hb	haemoglobin
HBB	Hank's binding buffer
HDC	heat denatured casein
HDS	haptoglobin depleted serum
Hip	Hsp70 interacting protein
HMW	high molecular weight
Hop	Hsp organizing protein
Hp	haptoglobin
HRP	horseradish peroxidase
Hsc	heat shock cognate
HSF	heat shock factor
Hsp	heat shock protein
Ig	Immunoglobulin
K _D	dissociation constant
LDLR	low density lipoprotein receptor
LRP	low density lipoprotein receptor related protein
Lys	lysozyme
α_2 M	α_2 -macroglobulin
α_2 M*	activated α_2 -macroglobulin
α_2 MDS	α_2 -macroglobulin depleted serum
β_2 M	β_2 -microglobulin
NADH	reduced nicotinamide adenine dinucleotide
NHS	normal human serum
NHS-LC-b	succinimidyl-6-[biotin-amido]hexanoate
OSB	oxidative stress buffer
Ovo	ovotransferrin
OX	oxidative
P	pellet
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline

PDAPP	PDGF promoter expressing amyloid precursor protein
PI	propidium iodide
PrP	prion protein
RAP	receptor associated protein
RT	room temperature
S	supernatant
SAP	serum amyloid P component
SaRIgG	sheep anti rabbit IgG
SDS	sodium dodecyl sulphate
SE	standard error
SEC	size exclusion chromatography
SH3	SH3 domain of the p85 alpha subunit of phosphatidylinositol 3 kinase
sHSP	small heat shock protein
SMR	subunit molar ratio
SOD	superoxide dismutase
$\tilde{\alpha}$ syn	α -synuclein
TEM	transmission electron microscopy
TGF β	transforming growth factor β
Thio T	thioflavin T
TNF α	tumor necrosis factor α
TPR	tetratricopeptide repeats
tRNA	transfer ribonucleic acid
UV	ultra violet
VLDLR	very low density lipoprotein receptor

List of Publications

Yerbury JJ, Rybchyn MS, Easterbrook-Smith SB, Henriques C, Wilson MR. (2005) The acute phase protein haptoglobin is a mammalian extracellular chaperone with an action similar to clusterin. *Biochemistry* 44: 10914-10925

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French K, **Yerbury JJ**, Wilson MR. (2008) Protease activation of α_2 -macroglobulin modulates a chaperone-like action with broad specificity. *Biochemistry* 47: 1176-1185.

Wilson MR, **Yerbury JJ**, Poon S. (2008) “Extracellular chaperones and amyloids” in the book titled “*Heat shock proteins and the brain: Implications for Neurodegenerative disease and Neuroprotection*”. Series: Heat Shock Proteins, Volume 3. Springer publications, New York, USA. Edited by Alexander Asea and Ian R. Brown.

Park DC, Yeo SG, Wong K, **Yerbury JJ**, Wilson MR, Bandera CA, Welch R, Choi YK, Birrer MJ, Berkowitz RS, and Mok SC. Overexpression of clusterin confers paclitaxel resistance in ovarian cancer. *Manuscript submitted*.

List of Conference Presentations

Poster presentation “Haptoglobin is an extracellular chaperone with an action similar to that of clusterin” at the 29th Annual Lorne Conference on Protein Structure and Function. Lorne, Victoria, Australia, February 8-12, 2004.

Oral Presentation “The effects of clusterin on amyloid formation” at the 4th International Workshop on Clusterin, Villars-sur-Ollon, Switzerland, June 16-18, 2005.

Poster presentation "Does the extracellular chaperone clusterin affect amyloidogenesis" at the FASEB Summer Research Conference entitled "Amyloid fibril formation, protein misfolding and aggregation" Snowmass Village, CO, USA, June 10-15, 2006.

Poster presentation “The extracellular protease inhibitor α_2 -macroglobulin has chaperone-like properties” at the World Conference of Stress, Budapest, Hungary 23-26 August 2007.

Invited oral presentation “Quality control of extracellular protein folding: An emerging field” at the World Conference of Stress, Budapest, Hungary 23-26 August 2007.

Abstract

Individual proteins have a specific three-dimensional structure that gives them their unique function. However, a protein must be folded from a linear string of amino acids in order to gain this native conformation and thus function. There are many hurdles to a protein attaining and maintaining its native conformation. Stresses that are encountered in the life of a protein, such as changes in pH, temperature and oxidative stress, can promote protein misfolding or unfolding. In addition, some genetic mutations can modify a protein such that a non-native conformation is more energetically favourable than the native state. The unfolding or misfolding of a protein makes it more likely that it will aggregate with itself. There are more than 40 human diseases associated with the inappropriate deposition of aggregated protein. It is well known that there is a well-defined and efficient quality control system to deal with intracellular proteins that have either unfolded or are misfolded. Cells have a range of molecular chaperones to inhibit inappropriate aggregation and if this fails the cell labels the proteins with ubiquitin for degradation via the proteasome. However, many proteins are secreted from cells into the extracellular environment and there are many protein deposition disorders associated with extracellular protein deposits, outside the reach of the well-characterised intracellular quality control system.

This thesis reports that the secreted proteins haptoglobin and α_2 -macroglobulin have small heat shock protein-like chaperone activity. Both haptoglobin and α_2 -macroglobulin specifically inhibited the precipitation of a variety of proteins induced by either heat or oxidation, including proteins in unfractionated human serum. In addition, it was demonstrated that haptoglobin and α_2 -macroglobulin inhibit the precipitation of stressed proteins by forming solubilized complexes with them, cannot protect enzymes from heat-induced loss of function, and lack ATPase activity and the ability to independently refold proteins following stresses. In addition, data presented here shows that clusterin, haptoglobin and α_2 -macroglobulin exert potent effects on amyloid formation, and provide evidence to suggest that these effects are exerted via interactions with pre-fibrillar species. These findings suggest that clusterin, haptoglobin and α_2 -macroglobulin are an important element in the control of extracellular protein misfolding.